

1 **Bioabsorbable Implantable Substrate**

2

3 The present invention relates to a method of
4 producing a bioabsorbable implantable substrate, a
5 method of altering the rate of bioabsorbability of
6 a least a portion of a bioabsorbable implantable
7 substrate, and a bioabsorbable implantable
8 substrate, having graded molecular weight
9 distribution formed according to these methods.

10

11 The long-term goal of biomaterials research lies in
12 tissue regeneration, not replacement. In 'tissue
13 engineering' biocompatible structures can be used
14 either to engineer *in-vitro* living cellular
15 constructs for transplantation, or to temporarily
16 support load and facilitate *in-vivo* mechanisms for
17 tissue regeneration. The ideal material for these
18 purposes should provide high strength initially,
19 then gradually degrade, transferring mechanical
20 loads to regenerating tissue. Typical surgical
21 applications are in the repair of connective soft
22 tissue, ligaments or tendons and hard tissue such
23 as bone.

1 In applications where tissue only requires
2 temporary support or fixation the use of
3 bioabsorbable polymers is appropriate. Depending on
4 the choice of material and processing conditions,
5 bioabsorbable polymers may retain their tissue
6 supporting properties for days, weeks or months.
7 Advantages of these materials are firstly, reduced
8 risk of long-term complications because stresses
9 are eventually transferred to the healing tissue,
10 and secondly, the avoidance of the necessity for a
11 retrieval operation.

12

13 Current trends in orthopaedic practice and
14 research suggest that the most important
15 bioabsorbable polymers used in surgery are
16 synthetic polymers such as aliphatic polyesters
17 (e.g. polyglycolide (PGA), polylactide (PLA) and
18 their copolymers). These polyesters degrade *in-*
19 *vivo* by hydrolysis into lactic acid and glycolic
20 acid, which are then incorporated in the
21 tricarboxylic acid cycle and excreted. These types
22 of polymer generally degrade by bulk erosion, as
23 the rate at which water penetrates the material
24 exceeds the rate at which chain scission (into
25 water-soluble fragments) occurs within the polymer
26 [Middleton, J.C., Tipton, A.J., *Biomaterials*,
27 2335-2346, 2000]. Degradation in the interior of
28 the device may occur faster than on the surface
29 due to autocatalysis. The implication of this is
30 that the device remains as a space-filler long
31 after the useful strength of the polymer has
32 deteriorated. The ingrowth of natural tissue is

1 prevented, and a 'lactide-burst' of low pH
2 material may be released when the surface of the
3 implant is finally degraded which can damage
4 surrounding cells and cause inflammation.

5

6 According to a first aspect of the present
7 invention there is provided a method of producing
8 a bioabsorbable, implantable substrate having a
9 graded molecular weight distribution, comprising
10 the steps of providing an implantable substrate
11 and altering the molecular weight distribution of
12 at least a portion of the implantable substrate by
13 exposing that portion of the implantable substrate
14 to electron beam irradiation.

15

16 Advantageously the molecular weight distribution
17 of the portion of the implantable substrate
18 exposed to electron beam irradiation is reduced.

19

20 Preferably at least a portion of the surface of
21 the implantable substrate is exposed to electron
22 beam irradiation. Suitably the molecular weight
23 distribution of the entire surface or body of the
24 implantable substrate is altered by exposing the
25 entire surface of the implantable substrate to
26 electron beam irradiation.

27

28 At least a portion of the implantable substrate
29 may be exposed to electron beam irradiation for
30 0.1 to 100 seconds; suitably for 1 to 90 seconds;
31 more suitably 3 to 60 seconds.

32

1 The electron beam irradiation may have an
2 intensity of 0.1 to 20 MeV; suitably 0.5 to 15
3 MeV; more suitably 1 to 5 MeV. A total radiation
4 dose of 1 to 100 kGy may be applied to the
5 implantable substrate. In one embodiment the
6 implantable substrate may be subject to more than
7 one dose of radiation; suitably 2 to 4 doses of
8 radiation. Each dose of radiation may be 1 to 50
9 kGy.

10

11 Suitably the electron beam irradiation penetrates
12 0.1 to 50 mm from the surface of the implantable
13 substrate; more suitably the electron beam
14 irradiation penetrates 2 to 20 mm. In one
15 embodiment the electron beam irradiation
16 penetrates 2 to 15 mm.

17

18 The implantable substrate may have a wall
19 thickness of at least 50 mm; suitably of 15 mm or
20 less; more suitably of 5 mm or less.

21

22 In one embodiment the flexural strength of the
23 portion of the implantable substrate exposed to
24 electron beam irradiation is altered; suitably
25 reduced.

26

27 In one embodiment the percentage mass loss of the
28 portion of the implantable substrate exposed to
29 electron beam irradiation is altered; suitably
30 reduced.

31

32

1 Preferably the exposure to electron beam
2 irradiation also causes sterilisation of the
3 implantable substrate.

4

5 The method may comprise the step of exposing the
6 implantable substrate to one or more doses of
7 electron beam irradiation. Each dose of electron
8 beam irradiation may be at a different intensity.

9

10 Suitably each dose of electron beam irradiation
11 penetrates the implantable substrate to a
12 different depth. The molecular weight
13 distribution, and thus the rate of biodegradation
14 of the implant is suitably different at different
15 depths.

16

17 According to a second aspect, the present
18 invention also provides a method of modifying the
19 rate of bioabsorbability of at least a portion of
20 a bioabsorbable, implantable substrate comprising
21 the step of exposing that portion to electron beam
22 irradiation.

23

24 According to a third aspect of the present
25 invention there is provided a bioabsorbable,
26 implantable substrate obtainable by either of the
27 methods described above.

28

29 According to a fourth aspect of the present
30 invention, there is provided a bioabsorbable
31 implantable substrate comprising a bioabsorbable
32 polymer having a graded molecular weight

1 distribution through at least a portion of its
2 thickness.

3

4 According to a fifth aspect of the present
5 invention, there is provided a bioabsorbable
6 implantable substrate having an outer surface and a
7 core wherein the molecular weight distribution of
8 the implantable substrate is greater at the core
9 than towards the outer surface, and the core is
10 less bioabsorbable than towards the outer surface.

11

12 Preferably the bioabsorbable implantable substrate
13 of the present invention is bioabsorbable at a
14 predetermined rate.

15

16 The implantable substrate of the present invention
17 may have a graded molecular weight distribution
18 through at least a portion of its thickness from
19 its surface thickness to the complete thickness of
20 the implantable substrate. The molecular weight
21 distribution of the implantable substrate may be
22 lower towards the surface, causing the rate of
23 bioabsorbability to be higher towards the surface.

24 The rate of bioabsorbability may be pre-determined
25 and controlled by altering the molecular weight
26 distribution of the implantable substrate. The
27 initial strength and average strength during
28 degradation of the implantable substrate of the
29 present invention are therefore also predictable
30 and controllable.

31

1 In one embodiment, the outer surface of the
2 implantable substrate biodegrades initially and
3 the load bearing strength of the substrate is
4 retained from the core. The implantable substrate
5 of the present invention thus allows the ingrowth
6 of natural tissue, whilst still providing some
7 structural support.

8

9 Preferably the outer surface and the core of the
10 bioabsorbable implantable substrate are formed from
11 the same material.

12

13 In general the bioabsorbable implantable substrate
14 is suitably formed from aliphatic polyesters such
15 as polyglycolide (PGA), polycaprolactone,
16 polylactide (PLA), poly(dioxanone) (PDO),
17 poly(glycolide-co-trimethylene carbonate) (PGA-
18 TMC), polyanhydrides, poly(propylene fumarate),
19 polyurethane and copolymers.

20

21 The molecular weight distribution of the substrate
22 is dependent on the material of the implantable
23 substrate, but suitably the molecular weight
24 distribution of the outer surface of the
25 implantable substrate is from 10,000 to 200,000 and
26 the molecular weight distribution of the core of
27 the implantable substrate is from 100,000 to
28 500,000. Preferably the molecular weight
29 distribution of the implantable substrate changes
30 gradually from the surface to the core.

31

1 The rate of absorption of the implantable substrate
2 into the body is dependant upon the material of the
3 implantable substrate and the size of the
4 implantable substrate. However, the rate of
5 absorption of the implantable substrate of the
6 present invention may preferably be pre-determined
7 and controlled to suit its purpose.

8

9 Preferably the implantable substrate is bioabsorbed
10 within 20 to 365 days, more preferably 60 to 120
11 days.

12

13 The bioabsorbable implantable substrate of the
14 present invention may comprise additives such as
15 bioactive agents and drugs. The additives may be
16 incorporated into the bioabsorbable polymer to
17 enhance tissue regeneration or reduce implant-
18 related infection. The rate of release of the
19 additives is not necessarily linear, and is
20 dependent upon the absorption rate of the polymers,
21 but is typically released over 20 to 175 days. The
22 bio-active agents are released in a controlled
23 manner as the outer surface of the implantable
24 substrate biodegrades, and later as the core
25 biodegrades. As such, the bio-active agents may be
26 released as and when required to enhance tissue
27 remodelling, and/or minimise the risk of infection.

28

29 Preferably the implantable substrate is an
30 interference screw, suture anchor, bioresorbable
31 polymer composite (which is suitably self-

1 reinforced), or a bioabsorbable scaffold for tissue
2 regeneration and growth.

3

4 The implantable substrate may cultivate tissue *in-*
5 *vivo* or *in-vitro*.

6

7 According to a sixth aspect of the present
8 invention there is provided the use of the
9 bioabsorbable implantable substrate hereinbefore
10 described, in the repair or treatment of disorders
11 of or damage to hard or soft tissue.

12

13 According to a seventh aspect of the present
14 invention there is provided a method of treatment
15 of a disorder of, or damage to hard or soft tissue
16 comprising the step of implanting the bioabsorbable
17 implantable substrate as hereinbefore defined in a
18 human or animal body.

19

20 According to an eighth aspect of the present
21 invention there is provided the bioabsorbable
22 implantable substrate as hereinbefore defined for
23 use in therapy.

24

25 Suitably the hard or soft tissue may be connective
26 tissue, ligaments, tendons or bone.

27

28 The disorder may be any tissue defect or trauma
29 including osteo or rheumatoid arthritis,
30 osteoporosis, inflammatory, neoplastic, traumatic
31 and infectious tissue conditions, syndromes
32 characterised by chondrodysplasia, cartilage

10

1 damage, fracture, ligament tears, hernia,
2 synovitis, systemic lupus erthematosus, or wounds,
3 particularly those sustained during surgery.

4

5 The degradation rate of bioabsorbable polymers is
6 at least partially dependent on their initial
7 molecular weight. The higher the initial molecular
8 weight the longer the bioabsorption time (if all
9 other factors are kept similar). It is now well
10 established that bioabsorbable polymers degrade by
11 essentially the same mechanism - hydrolytic
12 scission of the ester bonds. The reaction is
13 autocatalytic and follows pseudo first order
14 reaction kinetics:

15
$$M_n = M_{n,0} e^{-kt},$$

16

17 wherein:

18 M_n = molecular weight at a time from
19 implantation;

20 $M_{n,0}$ = initial molecular weight;

21 e = exponential function;

22 k = constant;

23 t = time from implantation.

24

25 k is suitably 1 to $9 \times 10^{-6} \text{ s}^{-1}$. k is typically
26 $1.16 \times 10^{-6} \text{ s}^{-1}$ for polyglycolides.

27

28 Therefore if the initial molecular weight of a
29 polymer is known, the degradation rate can be
30 predicted. The decrease in strength with time is
31 also predictable from the molecular weight, using
32 the equation:

$$1 \quad \sigma = \sigma_{\infty} - \frac{B}{M_n},$$

2 wherein:

3 σ = strength at a time (t) from implantation;

4 σ_{∞} = initial strength;

5 B = constant.

6

7 B is suitably 1 to 9×10^5 MPa g⁻¹ mol. B is
8 typically 3×10^5 MPa g⁻¹ mol for polyglycolides.

9

10 The penetration depth for electron beam irradiation
11 depends on the energy of the electrons used and the
12 density of the absorbing material. Penetration
13 depth can be predicted from the expression:

$$14 \quad d = \frac{(0.524E - 0.1337)}{\rho}$$

15

16 d = depth (cm);

17 E = electron energy (MeV);

18 ρ = density (gcm⁻³).

19

20 The typical densities of polyesters such as PGA and
21 PLLA are in the range 1.0-1.5 gcm⁻³, therefore
22 electron penetration depth for energies in the
23 range 0.3 to 10 MeV would be approximately 0.2 mm
24 to 40 mm. The energy of a 10 MeV electron beam
25 accelerator can be reduced by the use of metallic
26 shielding of various thicknesses.

27

28 The present invention will now be described by way
29 of example only, with reference to the accompanying
30 drawings in which:

1 Figure 1 is an illustration showing the
2 bioabsorption behaviour of an implantable substrate
3 known in the art wherein diagonal hatching
4 represents the degradation rate and molecular
5 weight of the substrate and increased width of
6 hatching indicates increased degradation rate and
7 decreased molecular weight and wherein horizontal
8 hatching represents fragmentation of the substrate;

9

10 Figure 2 is an illustration showing the
11 bioabsorption behaviour of an implantable substrate
12 according to the present invention wherein diagonal
13 hatching represents the degradation rate and
14 molecular weight of the substrate and increased
15 width of hatching indicates increased degradation
16 rate and decreased molecular weight and wherein
17 horizontal hatching represents fragmentation of the
18 substrate;

19

20 Figure 3 shows the flexural strength of implantable
21 substrates formed according to the method of
22 Example 1 at different depths from the surface of
23 the implantable substrate immediately after
24 exposure to e-beam irradiation (0 days) and after
25 exposure for 1 day to conditions which induce
26 accelerated degradation (1 day);

27

28 Figure 4 shows the polystyrene molecular weight
29 equivalent (M_w) and the average molecular weight
30 (M_n) of implantable substrates formed according to
31 the method of Example 1 at different depths from
32 the surface of the implantable substrate;

1 Figure 5 shows the percentage mass loss of
2 implantable substrates formed according to the
3 method of Example 1 at different depths from the
4 surface of the implantable substrate after exposure
5 for 12 days to conditions which induce accelerated
6 degradation.

7

8 Figure 1 shows that upon implantation in a human or
9 animal body known implantable substrates undergo a
10 loss in strength and mass across their entire
11 cross-section. Known implantable substrates have
12 an even molecular weight distribution across their
13 thickness and so the core and surface of known
14 implantable substrates are bioabsorbed at
15 approximately the same rate. The space occupied by
16 known implantable substrates does not reduce until
17 the known implant is almost entirely bioabsorbed.

18

19 After implantation for a prolonged period of time,
20 known implantable substrates undergo fragmentation
21 due to a loss in mass. The core of such an
22 implantable substrate fragments before the surface
23 which may result in a "lactide-burst" of low pH
24 material which can damage surrounding cells and
25 cause inflammation.

26

27 Figure 2 shows an implantable substrate according
28 to the present invention, and shows how the
29 implantable substrate is bioabsorbed upon
30 implantation into a human or animal body. The
31 implantable substrate of the present invention has
32 a graded molecular weight distribution, wherein the

1 surface of the implantable substrate has a lower
2 molecular weight distribution than the core.

3

4 The surface of the implantable substrate is
5 bioabsorbed at a faster rate than the core of the
6 implantable substrate, such that the surface of the
7 implantable substrate undergoes loss in strength
8 before the core and the space occupied by the
9 implantable substrate is reduced gradually, thus
10 allowing greater tissue ingrowth into the space
11 occupied by the implant.

12

13 The core of the implantable substrate may still
14 fragment but is bioabsorbed after the surface of
15 the implantable substrate. The space occupied by
16 the implantable substrate is reduced gradually
17 during bioabsorption, encouraging tissue ingrowth.

18

19 Example 1

20 Poly(L-lactide) PLLA was moulded into sheets with a
21 thickness of approximately 0.9 mm in a Collin P 200
22 P platen press at temperatures increasing to 200 °C
23 and pressures increasing to 100 bar. The PLLA used
24 was from Resomer® L (Batch Number 26033), supplied
25 in pellet form by Boehringer Ingelheim (Ingelheim,
26 Germany). Gel Permeation Chromatography of the
27 material gave the molecular weight as 462,000
28 (expressed as polystyrene molecular weight
29 equivalent) and the Mn number (average molecular
30 weight) as 166,000 (expressed as polystyrene
31 molecular weight equivalent). The sheets were then
32 manufactured into ISO 527-2 standard tensile

1 samples approximately 75 mm in length using a hand
2 operated table press. The samples were then
3 annealed in an oven at 120 °C for 4 hours to give a
4 more realistic representation of processed
5 material.

6

7 In order to study the effects of e-beam radiation
8 on the PLLA materials at different depths within a
9 bulk of material, spacers with similar material
10 properties to PLLA were required. Sheets of
11 acrylic having a similar density to the PLLA
12 samples were chosen. The samples and the acrylic
13 sheet spacers were stacked and 28 tensile PLLA
14 samples were irradiated at 5 different depths;
15 namely 0 mm, 3.9 mm, 13.9 mm, 27.3 mm and 42.7 mm
16 from the surface of the composite structures. The
17 stacked samples and spacers were framed by acrylic
18 sheets with a wall thickness of at least 50 mm.
19 This ensured that radiation reached the PLLA
20 samples from the intended direction only. The
21 samples were then irradiated at Ebis Iotron
22 (Didcot, Oxfordshire) using a 10 MeV electron beam
23 machine. The radiation dose was set to give the
24 upper surface of the composite sample, and
25 therefore the 0 mm depth PLLA samples, a radiation
26 dose of 40 kGy. The samples were stored in a
27 desiccator cupboard following irradiation.

28

29 The medium used for the *in vitro* degradation of the
30 PLLA samples was a "Sörensen" pH 7.4 buffer
31 solution prepared from potassium
32 dihydrogenphosphate (KH_2PO_4) and disodium

1 hydrogenphosphate (Na_2HPO_4). These salts were
2 mixed into a solution in a ratio of 1:15 mol/l.
3 The solutions were then combined at a ratio of
4 18.2% KH_2PO_4 solution and 81.8% Na_2HPO_4 solution.
5 This ratio is set out by ISO 15814: "Implants for
6 surgery - Copolymers and blends based on
7 polylactide - *In Vitro* degradation testing". Each
8 tensile sample of PLLA material was weighed before
9 being placed in a vial with approximately 20ml of
10 buffer solution. The vials were then placed in an
11 oven at 70°C. At specified time periods, 5 samples
12 from each depth were removed, and then blot dried
13 and weighed for water uptake measurements. The
14 samples were then tensile tested using an Instron
15 Universal materials testing machine in accordance
16 with ISO 527-2. After testing, the samples were
17 dried and weighed to obtain mass loss results. Gel
18 Permeation Chromatography was carried out on the
19 tested samples to determine the molecular weight of
20 the degraded PLLA. The results were compared to a
21 control sample which had not been exposed to e-beam
22 irradiation.

23

24 The irradiated samples were subject to temperatures
25 of up to 70°C for one day to induce accelerated
26 degradation and the flexural strength of the
27 samples were recorded immediately after e-beam
28 irradiation and after accelerated degradation had
29 been induced. The results were compared to a
30 control sample which had not been exposed to e-beam
31 irradiation.

32

1 A mass-loss study was designed to determine how the
2 irradiation had affected the resorption rate of the
3 polymer. To assess this accelerated degradation
4 was induced. To allow four time points, with three
5 repetitions at each, 12 samples were prepared for
6 each cross-sectional depth and for the control.
7 Each sample weighed approximately 0.085g. The
8 samples were dried in a vacuum oven at 37°C for 48
9 hours before being individually weighed, and their
10 masses recorded. The samples were then placed in
11 "Sörensen" pH 7.4 buffer-solution, as described
12 previously, and stored in an oven at 70°C. After
13 set periods of times three samples from each set
14 were removed from the oven. The samples and buffer
15 solution were filtered using hardened ashless
16 filter paper. The filtrate was then rinsed with
17 deionised water and re-filtered. The filter paper
18 containing the filtrate was then dried in an oven
19 at 80°C for at least 3 hours before being cooled to
20 room temperature. The dried filtrate was then
21 removed and weighed. Through comparison of the
22 mass of the dried filtrate with the original mass
23 of the sample, the percentage mass loss was
24 determined. A control sample which had not been
25 exposed to e-beam irradiation was also analysed.

26
27 The results of the flexural strength tests are
28 summarised in Figure 3. Upon exposure to e-beam
29 irradiation the flexural strength towards the
30 surface of the sample (0 to 27.3 mm) was reduced.
31 The flexural strength at the core (i.e. 42.7 mm
32 from the surface) was approximately the same as the

1 flexural strength of the control sample and this
2 may suggest that the e-beam irradiation did not
3 penetrate to the core of the sample. The flexural
4 strength of all samples decreased after accelerated
5 degradation had been induced. The flexural
6 strength of samples at the core (42.7 mm from the
7 surface) remained approximately the same as the
8 flexural strength of the control sample after
9 accelerated degradation. The flexural strength
10 results suggest that implantable substrates exposed
11 to e-beam irradiation would have a tendency to
12 biodegrade gradually from the surface inwards.

13

14 The results of the molecular weight tests are
15 summarised in Figure 4. A control sample which had
16 not been exposed to any e-beam irradiation was also
17 analysed. Two measures of molecular weight were
18 taken from the samples: polystyrene molecular
19 weight equivalent (M_w) and average molecular weight
20 (M_n). Upon exposure to e-beam irradiation the
21 molecular weight (both M_w and M_n) of the
22 implantable substrate was reduced at depths of 3.9
23 to 27.3 mm from the surface. The molecular weight
24 at the core (i.e. 42.7 mm from the surface)
25 remained approximately the same as the molecular
26 weight of the control and this may suggest that the
27 e-beam irradiation did not penetrate to the core of
28 the sample. The molecular weight at the surface (0
29 mm) was unexpectedly high after exposure to e-beam
30 irradiation. This suggests that the implantable
31 substrate may have been exposed to too high a dose
32 of e-beam irradiation and that this may have

1 induced some cross-linking of the polymer at the
2 surface thus increasing the molecular weight at the
3 surface. The molecular weight results suggest that
4 implantable substrates exposed to e-beam
5 irradiation have a graded molecular weight
6 distribution from the surface to the core, the
7 molecular weight being greatest at the core.

8 Implantable substrates exposed to e-beam
9 irradiation would have a tendency to biodegrade
10 gradually from the surface inwards, reducing the
11 space occupied by the implantable substrate
12 gradually. However, if too high a dose of e-beam
13 irradiation is used cross-linking of the substrate
14 polymer may be induced at the surface leading to a
15 relatively high molecular weight at the surface.
16 This effect may be avoided by reducing the dose of
17 e-beam irradiation used.

18

19 Figure 5 summarises the results of the mass loss
20 tests. Upon exposure to e-beam irradiation the
21 percentage mass loss towards the surface (0 to 27.3
22 mm) was increased compared to the control. The
23 percentage mass loss of the surface was lower than
24 the percentage mass loss at slightly greater
25 depths. This may suggest that the dose of e-beam
26 irradiation was too high and induced some degree of
27 cross-linking on the surface. This was also
28 suggested by the molecular weight analysis. The
29 percentage mass loss of the core (42.7 mm) is
30 approximately the same as the percentage mass loss
31 of the control and this may suggest that the e-beam
32 irradiation did not penetrate the core of the

1 sample. The mass loss results indicate that
2 implantable substrates exposed to e-beam
3 irradiation would have a tendency to biodegrade
4 gradually from the surface inwards.